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The Adiponectin variants contribute to the genetic background of type 2 diabetes in Turkish population

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ABSTRACT

Adiponectin, an adipose tissue specific protein encoded by the Adiponectin gene, modulates insulin sensitivity and plays an important role in regulating energy homeostasis. Many studies have shown that single nucleotide polymorphisms (SNPs) in the Adiponectin gene are associated with low plasma Adiponectin levels, insulin resistance and an increased risk of type 2 diabetes mellitus. The aim of the present study was to evaluate the contribution of the Adiponectin gene polymorphisms in genetic background of type 2 diabetes in a Turkish population. In total, 169 unrelated and non-obese diabetic patients and 119 age- and BMI-matched non-diabetic individuals with no family history of diabetes were enrolled in this study. We detected a significant association between type 2 diabetes and two SNPs: SNP −11391G>A, which is located in the promoter region of the Adiponectin gene, and SNP +276G>T, which is found in intron 2 of the gene ($P<0.05$). The silence SNP G15G (+45T>G) in exon 1 and SNP +349A>G in intron 2 also showed a weak association with type 2 diabetes ($P=0.06$ and $P=0.07$, respectively), while SNPs −3971A>G in intron 1 and Y111H, R112C and H241P in exon 3 showed no association ($P>0.05$). In conclusion, these findings suggest that Adiponectin gene polymorphisms might be effective on susceptibility for type 2 diabetes development which emerged from the interactions between multiple genes, variants and environmental factors.

1. Introduction

Adipose tissue, initially, has been considered as a simple energy storing tissue, nowadays in addition to this function known as the largest endocrine organ of the body, with the capacity of secreting several biologically active polypeptides (Kim and Moustaid-Moussa, 2000). These polypeptides, termed as adipokines, including tumor necrosis factor-α (TNF-α), plasminogen activating inhibitor-1 (PAI-1), retinol binding protein-4 (RBP4), interleukin-6, leptin, resistin and Adiponectin, play multiple roles that effect the metabolism and energy balance, insulin sensitivity, inflammation and atherogenesis (Ahima and Flier, 2000; Chandran et al., 2003; Galic et al., 2010; Tomas et al., 2002; Uysal et al., 1997; Whitehead et al., 2006).

Adiponectin, predominantly expressed by adipose tissue, is an abundant plasma protein (500–300,000 μg/l) (Hu et al., 1996), accounting for 0.01% of total protein (Ouchi et al., 2003). Importantly, contrary to other adipokines, Adiponectin plasma levels are lower in patients with type 2 diabetes mellitus (T2DM) (Gibson and Froguel, 2004; Li et al., 2009), obesity (Arita et al., 1999; Yang et al., 2002a), insulin resistance (Hotta et al., 2000; Weyer et al., 2001; Yang et al., 2002b), dyslipidemia (Matsubara et al., 2002; Yang et al., 2002b) and cardiovascular diseases (Hotta et al., 2000; Kumada et al., 2003).

The Adiponectin protein, which belongs to the complement factor C1Q family, is composed of 244 amino acids and approximately 30 kilodalton (kDa). It contains an N-terminal signal sequence, a variable domain, a short collagen-like domain composed of Gly-X-Y repeats, and a large C-terminal globular domain of approximately 140 amino acids (Hotta et al., 2000; Hu et al., 1996; Shapiro and Scherer,

Abbreviations: T2DM, type 2 diabetes mellitus; SNPs, single nucleotide polymorphisms; BMI, body mass index; GWA, genome wide association; TNF-α, tumor necrosis factor-α; PAI-1, plasminogen activating inhibitor-1; RBP4, retinol binding protein-4; HMW, high molecular weight; KCNJ11, potassium inwardly-rectifying channel subfamily J member 11; TCF7L2, transcription factor 7 like-2; PPAR, peroxiome proliferators-activated receptor; OGTT, oral glucose tolerance test; HOMA-IR, homeostasis model assessment of insulin resistance; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; HWE, Hardy–Weinberg equilibrium; OR, Odds ratio; LD, Linkage Disequilibrium; YY1, Yin Yang 1; C/EBP, CCAAT/enhancer binding protein; SREBP, sterol regulatory element binding protein; AMPK, 5′-AMP-activated protein kinase; NF, nuclear factor; MAP, mitogen-activated protein.

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1998). Adiponectin circulates through plasma in different forms via disulfide bond formation mediated by cystein residues situated the N-terminal domain, such as trimer, hexamer, and high molecular weight (HMW) form containing 12–18 molecules (Richards et al., 2006; Waki et al., 2003). In several studies it was shown that the most bioactive form of Adiponectin is HMW form (Wang et al., 2008), and this form has the most potent insulin sensitizing activity (Kadowaki et al., 2006; Scherer, 2006).

The Adiponectin gene is located on chromosome 3q27 and spans approximately 16 kilobases (kb) of DNA in a region identified as a susceptibility locus for type 2 diabetes. Several association studies strongly showed that the common single nucleotide polymorphisms (SNPs) within the Adiponectin locus, especially SNPs −11391G > A, −11377C > G, +45T > G, and +276G > T, determine alteration in plasma Adiponectin concentrations (Filippi et al., 2004; Gonzales-Sanchez et al., 2005; Menzaghi et al., 2002; Schwarz et al., 2006; Supriyaprom et al., 2010; Vasseur et al., 2002, 2005; Yang et al., 2008) and also subsequently several meta-analyses supported these findings (Dastani et al., 2012; Heid et al., 2010; Richards et al., 2009). These data and reports of studies showing association between Adiponectin variants and T2DM (Biswas et al., 2011; Hara et al., 2002; Vasseur et al., 2002, 2005; Yang et al., 2008; Zacharova et al., 2005) suggest Adiponectin gene to be a reasonable candidate gene for T2DM susceptibility. However, in last years, Genome Wide Association (GWA) studies on T2DM genetics (Scott et al., 2007; Sladek et al., 2007; Zeggini et al., 2008), surprisingly confirmed only a few genes, suggested as in relation with T2DM by candidate gene approach such as potassium inwardly-rectifying channel subfamily J member 11 (KCNJ11), transcription factor 7 like-2 (TCF7L2) and peroxiome proliferators-activated receptor (PPAR) γ, but Adiponectin was not among them. On the other hand, due to the biological function of Adiponectin in adipokine signaling, glucose regulation and fatty acid metabolism, association and functional studies to display the effect of Adiponectin on T2DM are still continuing.

There are many association studies from different populations analyzing Adiponectin gene affect on type 2 diabetes but there is not any report whether Adiponectin gene is associated with T2DM from our country. We therefore investigated the role of Adiponectin gene polymorphisms in type 2 diabetes in a Turkish population.

2. Material and method

2.1. Clinical samples

Our study included 169 unrelated, non-obese patients from the Endocrinology Department of Meram Medical Faculty and the Konya Diabetes Society, Turkey, who were diagnosed as type 2 diabetics according to the American Diabetes Association diagnostic criteria. Individuals in the diabetic group were over 35 years old, did not use insulin, and had a body mass index (BMI) of less than 30. In healthy group, age- and BMI-matched 119 non-diabetic individuals with no family history of diabetes were included in this study. Informed written consent was obtained from each individual before participation into the study. The study was approved by the Ethical Committee of the Meram Medical Faculty.

2.2. Clinical analyses

Fasting plasma glucose, fasting insulin, HbA1C and c-peptide values were measured for both diabetic and control groups. In control group, oral glucose tolerance test (OGTT) was performed. Insulin resistance was detected by the homeostasis model assessment of insulin resistance (HOMA-IR) and calculated as fasting plasma glucose (mmol/l) multiplied by fasting serum insulin (pmol/l) and divided by 22.5. An individual with a HOMA-IR value higher than 2.5 was considered to be resistant to insulin (Duncan et al., 1995).

2.3. Genotyping

Genomic DNA was isolated from peripheral blood leukocytes using standard proteinase K and SDS procedure. The nucleotide sequence of the Adiponectin gene was obtained from the GenBank™ database. Primers were designed to screen nine SNPs and polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) technique was utilized for SNP scanning. Genotyping was made taking into account the positive strand. Primer sequences are available on request.

PCR amplification was carried out in a 15 μl volume containing 50–100 ng genomic DNA, 1X PCR buffer, 0.4 mM of each primer, 0.6 mM deoxynucleoside triphosphates and 0.1 units Taq polymerase. PCR reactions were performed as follows: an initial denaturation at 94 °C for 5 min was followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at different temperatures for each primer for 30 s, elongation at 72 °C for 30 s, and a final extension at 72 °C for 2 min.

2.4. RFLP analyses

The PCR products of the target SNPs were digested with restriction enzymes according to the manufacturers' instructions. The restriction enzymes used are presented in Table 1. The resultant restriction fragments were ascertained on a 2.5–3% agarose gel and then visualized.

2.5. Sequencing

The results of the RFLP analyses and subsequent agarose gel electrophoresis experiments were confirmed with sequencing by Iontek AS (Istanbul, Turkey). The DYEnamic ET terminator Cycle Sequencing Kit (Amersham Biosciences Piscataway, NJ, USA) and ABI PRISM 310 Genetic Analyzer were used according to instructions provided by the manufacturers. Sequence traces were evaluated individually and alignments were compared to sequences available in GenBank™ using the NCBI blast program.

2.6. Statistical analysis

Descriptive statistics were obtained for clinical and biochemical characteristics. Initial comparisons were performed between patient and control groups using *t*-test. Chi-square goodness of fit test was then utilized to evaluate Hardy–Weinberg equilibrium (HWE) in patient and control groups. Analyses were carried out using dominant, additive, and recessive models. Dominance was defined in terms of allele 2 effects. In dominant allele 2 models, homozygous individuals for allele 1 were compared with carriers of allele 2. In recessive allele 2 models, homozygous individuals for allele 2 were compared with carriers of allele 1. If no individual exist in one of the homozygous genotype, existing homozygous genotype was compared with heterozygous genotype. Allele frequencies of SNPs in patient and control groups were evaluated using Odds ratio (OR). Further, patient and control group's ORs were obtained for pair-wise SNP genotypes for dominant,

Table 1
Characteristics of studied SNPs in Adiponectin gene.

SNP no	SNP name	Location	Type	Base change	Enzyme
rs17300539	−11391	Promoter	Noncoding	G → A	*Hpa*II
rs822396	−3971	İntron 1	Noncoding	A → G	*Mse*I
rs2241766	G15G	Exon 2	Silent	T → G	*Sma*I
rs1501299	+276	İntron 2	Noncoding	T → G	*Bsm*I
rs2241767	+349	İntron 2	Noncoding	A → G	*Bsu*36I
rs62625753	G90S	Exon 3	Missense	G → A	*Ava*II
rs17366743	Y111H	Exon 3	Missense	T → C	*BstZ*17I
rs121917815	R112C	Exon 3	Missense	C → T	*BsrB*I
rs141205818	H241P	Exon 3	Missense	A → C	*Nla*III